



NICEATM-ICCVAM Update

**William S. Stokes, D.V.M, D.A.C.L.A.M.
RADM, U.S. Public Health Service
Executive Director, ICCVAM
Director, NICEATM**

**Meeting of the Scientific Advisory Committee
on Alternative Toxicological Methods**

June 25-26, 2009

Arlington, VA



Acknowledgements: Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Consumer Product Safety Commission

♀ Marilyn Wind, Ph.D. (Chair)
♂ Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture

♀ Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
♂ Elizabeth Goldentyer, D.V.M.

Occupational Safety and Health Administration

♀ Surender Ahir, Ph.D.

Department of Energy

♀ Michael Kuperberg, Ph.D.
♂ Marvin Stodolsky, Ph.D.

Department of the Interior

♀ Barnett A. Rattner, Ph.D.

Department of Transportation

♀ George Cushmac, Ph.D.
♂ Steve Hwang, Ph.D.

National Cancer Institute

♀ T. Kevin Howcroft, Ph.D.
♂ Chand Khanna, D.V.M., Ph.D.

National Institutes of Health

♀ Margaret D. Snyder, Ph.D.

♀ Principal Agency Representative

♂ Alternate Principal Agency Representative

Environmental Protection Agency

Office of Pesticide Programs

♀ Jack Fowle, Ph.D.
♂ Tina Levine, Ph.D.
♂ Vicki Dellarco, Ph.D.
Deborah McCall

OECD Test Guidelines Program

Christine Augustyniak, Ph.D.

Office of Research and Development

Julian Preston, Ph.D.
Stephanie Padilla, Ph.D.

Agency for Toxic Substances and Disease Registry

♀ Moiz Mumtaz, Ph.D.
♂ Bruce Fowler, Ph.D.

National Institute of Environmental Health Sciences

♀ William S. Stokes, D.V.M., D.A.C.L.A.M.
♂ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., D.A.B.T.
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health

♀ Paul Nicolaysen, V.M.D.
♂ K. Murali Rao, M.D., Ph.D.

Department of Defense

♀ Robert E. Foster, Ph.D.
♂ Patty Decot
Peter J. Schultheiss, D.V.M., D.A.C.L.A.M.
Harry Salem, Ph.D.

Food and Drug Administration

Office of the Commissioner

♀ Suzanne Fitzpatrick, Ph.D., D.A.B.T.
Center for Drug Evaluation and Research

♂ Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.

Center for Devices and Radiological Health

Melvin E. Stratmeyer, Ph.D.
Vasant Malshet, Ph.D., D.A.B.T.

Center for Biologics Evaluation and Research

Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.

Center for Food Safety and Nutrition
David G. Hattan, Ph.D.

Robert L. Bronaugh, Ph.D.

Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.

National Center for Toxicological Research

Paul Howard, Ph.D.
Donna Mendrick, Ph.D.

Office of Regulatory Affairs
Laurence A. D'Hoostelaere, Ph.D.

National Library of Medicine

♀ Pertti Hakkinen, Ph.D.
♂ Jeanne Goshorn, M.S.



Acknowledgements: NICEATM

NIEHS

William S. Stokes, D.V.M., D.A.C.L.A.M.

Debbie McCarley

Director; Project Officer

Special Assistant; Asst. Project Officer

Center Support Contract (ILS, Inc.)

David Allen, Ph.D., *Principal Investigator*

Jonathan Hamm, Ph.D.

Eleni Salicru, Ph.D.

Brett Jones, Ph.D.

Nelson Johnson

James Truax, M.S.

Frank Stack

Catherine Sprankle

Judy Strickland, Ph.D., D.A.B.T.

Elizabeth Lipscomb, Ph.D.

Frank Deal, M.S.

Michael Paris

Patricia Ceger, M.S.

Thomas Burns, M.S.

Linda Wilson

Linda Litchfield



Updates

- NICEATM-ICCVAM Five-Year Plan
- International Cooperation on Alternative Test Methods
- Allergic Contact Dermatitis Assays
- Ocular Safety Assessments
- Acute Systemic Toxicity
- Endocrine Disruptor Assays
- Genetic Toxicity Assays
- Dermal Safety Assessment Assays
- Alternative Methods for Biologics Testing
- Outreach Activities



NICEATM-ICCVAM Five-Year Plan: 2008-2012




- Areas of Emphasis:
 - Priority test method activities
 - Application of new science and technology
 - Partnerships
 - International cooperation and harmonization
- Implementation Plan Developed
 - ICCVAM Implementation Plan Subcommittee
 - R&D Working Group
 - Agenda item today
- New Website Milestones Page
 - Track progress and activities



NICEATM-ICCVAM: Alternative Test Method Project Milestones


Search NICEATM-ICCVAM

search




National Toxicology Program
Department of Health and Human Services

[Home](#) [Testing Information](#) [Study Results & Research Projects](#) [Public Health](#) [About the NTP](#) [Help](#)



NICEATM-ICCVAM Home >> Test Method Evaluations >> Project Milestones



Alternative Test Method Project Milestones

This page provides a current summary of the status of ongoing and completed NICEATM-ICCVAM alternative test method evaluation projects, as well as projects to which NICEATM, ICCVAM, and agency scientists are contributing.

Alternative test methods:

- **Reduce** the number of animals used to the minimum number required to obtain scientifically valid data
- **Refine** procedures to lessen or eliminate animal pain and distress
- **Replace** animals with non-animal systems or one animal species with a less highly developed one (for example, replacing a mouse with a fish).

[More information about the ICCVAM Test Method Evaluation Process](#)

View status of test method evaluation projects grouped by project area


Acute Oral Systemic Toxicity
Biologics
Dermal Corrosivity
Dermal Irritation
Developmental Toxicity

Endocrine Disruptor
Genetic Toxicity
Immunotoxicity
Ocular Toxicity
Pyrogenicity

Select project name in summary table to jump down the page to details and links

Test Method	R&D	Validation Studies	Evaluation	Recommendations for U.S. Agencies	U.S. Acceptance	International Acceptance	Additional Activities
Up-and-Down Procedure	✓	✓	✓	✓	2002	2002	
Fixed Dose Procedure	✓	✓	✓	✓	2002	2002	
Acute Toxic Class Method	✓	✓	✓	✓	2002	2002	

[NICEATM-ICCVAM Home](#)
[Meetings and Workshop Schedule](#)
[Alternative Test Method Evaluations](#)
[Acute Oral Toxicity](#)
[Biologics and Vaccines](#)
[Dermal Corrosivity and Irritation](#)
[Developmental Toxicity](#)
[Endocrine Disruptor](#)
[Genetic Toxicity](#)
[Immunotoxicity](#)
[Ocular Toxicity](#)
[Pyrogenicity](#)
[Nominations and Submissions](#)
[Testing Regulations and Guidelines](#)
[Search NICEATM-ICCVAM Documents](#)
[Search Federal Register](#)


Site Map

6

NICEATM-ICCVAM: Advancing Public Health and Animal Welfare

ICCVAM

NICEATM

International Cooperation on Alternative Test Methods (ICATM)

■ Memorandum of Cooperation

- Signed at NIH: April 27, 2009

■ Signatories

- Dr. Linda Birnbaum, Director, NIEHS and NTP, USA
- Dr. Nishijima, Director, National Institute of Health Sciences, Japan
- Dr. Elke Anklam, Director, Institute of Consumer Protection and Health, JRC, European Commission
- Dr. David Blakey, Director, Health and Safety Bureau, Health Canada



■ Initial Participating Validation Organizations

- JaCVAM
- ECVAM
- Health and Safety Bureau, Health Canada
- NICEATM-ICCVAM



International Cooperation on Alternative Test Methods (ICATM)

- A framework for enhanced international cooperation, collaboration, and communication
- Three critical areas:
 1. Test method validation studies
 2. Independent peer review of the validation status of test methods
 3. Development of harmonized formal test method recommendations for regulatory authorities
- Goal: Accelerate international adoption of scientifically valid alternative test methods



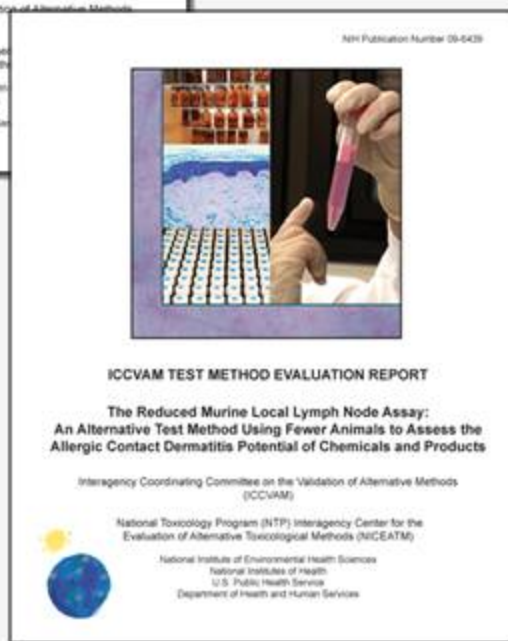
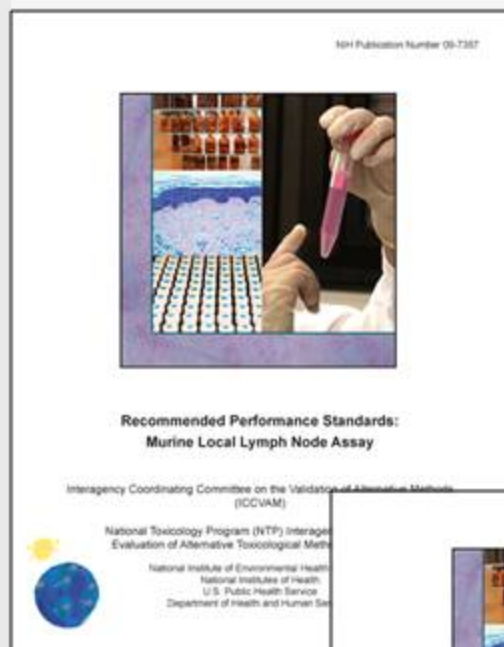
Alternative Methods for Allergic Contact Dermatitis

- ICCVAM Immunotoxicity Working Group
 - ECVAM liaison: Silvia Casati
 - JaCVAM liaison: Hajimi Kojima
- March 4-6, 2008 Peer Review Meeting
 - New versions and applications of the LLNA
- Updating OECD TG 429 (LLNA):
 - LLNA Performance Standards
 - Reduced LLNA procedure
 - Updated procedures
 - Dose-setting
 - Minimum number of animals: four per dose
- Coordinating with ECVAM-led validation studies
 - Peptide reactivity assays, *in vitro* assays



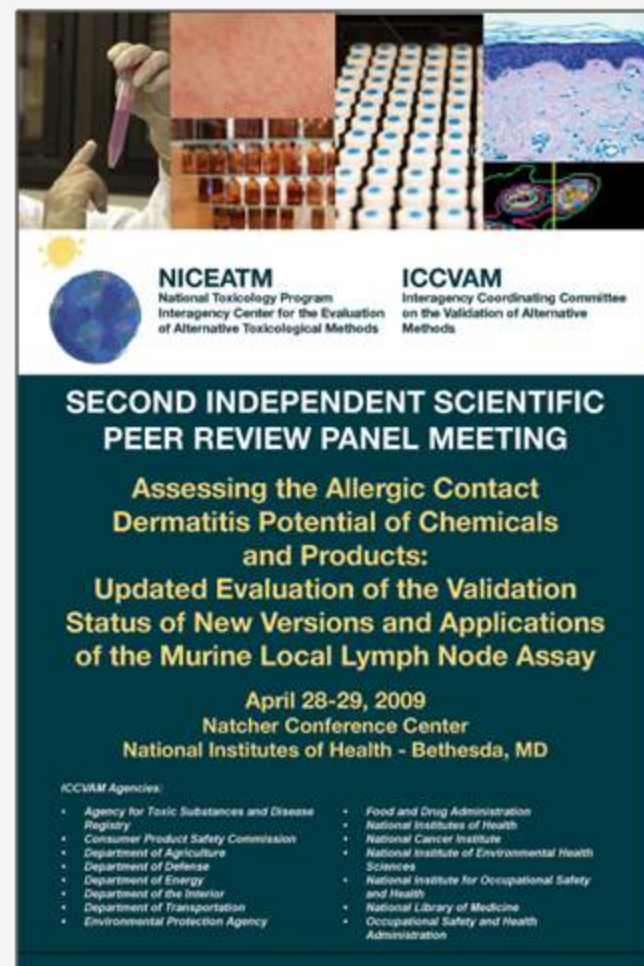
ICCVAM Test Method Evaluation Reports: LLNA

- **Final Recommendations**
 - LLNA Performance Standards
 - Reduced LLNA Procedure
 - Updated LLNA Protocol
- Forwarded to HHS Secretary for transmittal to agencies
- ICCVAM Evaluation Reports available online



International Peer Review Panel Meeting: LLNA New Versions and Applications

- April 28-29, 2009, NIH Natcher Conference Center, Bethesda, MD
 - Public meeting
- Evaluated updated data:
 - Three non-radioactive versions of the LLNA
 - Expanded LLNA applications
- Final Panel report published, June 1
 - Available on NICEATM-ICCVAM website
 - Public comments invited
 - **SACATM agenda item**
- Final ICCVAM Recommendations, Fall 2009



<http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm>



ICCVAM Ocular Safety Testing Peer Review Panel Meeting

NICEATM
National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM
Interagency Coordinating Committee on the Validation of Alternative Methods

**INDEPENDENT SCIENTIFIC
PEER REVIEW PANEL MEETING**

**Evaluation of the Validation Status of
Alternative Ocular Safety Testing Methods
and Strategies**

- Using topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress
- The *in vivo* low volume eye test
- Four *in vitro* test methods to identify moderate, mild, and nonlabeled ocular irritant categories
- *In vitro* strategy to assess the eye irritation potential of antimicrobial cleaning products

May 19-21, 2009
**Consumer Product Safety Commission
Headquarters - Bethesda, MD**

ICCVAM Agencies:

- Agency for Toxic Substances and Disease Registry
- Consumer Product Safety Commission
- Department of Agriculture
- Department of Defense
- Department of Energy
- Department of the Interior
- Department of Transportation
- Environmental Protection Agency
- Food and Drug Administration
- National Institutes of Health
- National Cancer Institute
- National Institute of Environmental Health Sciences
- National Institute for Occupational Safety and Health
- National Library of Medicine
- Occupational Safety and Health Administration

NIEHS
National Institute of Environmental Health Sciences

NTP
National Toxicology Program

- May 19-21, 2009
 - CPSC Headquarters, Bethesda, MD
 - Open to the public
- Expert Scientific Panel
 - 22 scientists, 6 countries
- Evaluated 10 alternative methods and strategies
 - Routine use of analgesics and topical anesthetics
 - Low Volume Eye Test
 - *In vitro* methods and strategies
- Documents available to public
 - Draft Background Review Documents
 - Draft ICCVAM Recommendations
 - Public comments
- Agenda item today

Other Ocular Toxicity Test Method Activities

- **OECD Test Guidelines for BCOP and ICE**
 - Submitted by ICCVAM: July, 2008
 - OECD Expert Consultation Meeting
 - Washington D.C., December, 2008

- **Four cell function-based assays**
 - Cytosensor[®], Fluorescein Leakage, Neutral Red Release, and the Red Blood Haemolysis Assays
 - ECVAM BRDs, ESAC peer review in progress
 - ICCVAM draft recommendations provided
 - Cytosensor[®] considered by ICCVAM Peer Panel

- **ICCVAM OTWG:**
 - ECVAM Liaisons : João Barroso, Valerie Zuang, Tom Cole
 - JaCVAM Liaisons: Hajime Kojima

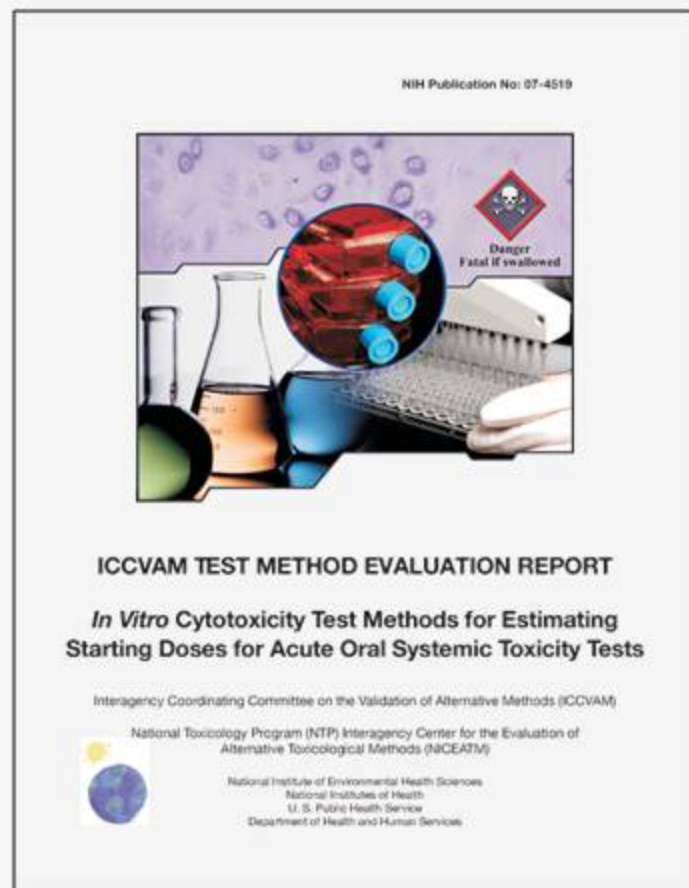


Acute Toxicity Test Method Activities

- Proposed OECD Test Guidelines and Guidance Documents: ICCVAM comments provided
 - TG 436: Acute Inhalation Toxicity – Acute Toxic Class Method
 - GD 39: Acute Inhalation Toxicity Testing
 - Revised TG 403: Acute Inhalation Toxicity
 - OECD Threshold Approach for Acute Fish Toxicity Testing
 - Supplement to TG 203: Fish, Acute Toxicity Test



Acute Toxicity Test Method Activities



- *In Vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Test*
- Final ICCVAM recommendations
 - All agencies concurred where applicable to their agency
- OECD Guidance Document in preparation



Acute Toxicity Test Method Activities

**Scientific Workshop on Acute Chemical Safety Testing:
Advancing *In Vitro* Approaches and Humane Endpoints
for Systemic Toxicity Evaluations**

NICEATM
National Toxicology Program Interagency Center for
the Evaluation of Alternative Toxicological Methods

ICCVAM
Interagency Coordinating Committee
on the Validation of Alternative Methods

ECVAM - European Centre for the
Validation of Alternative Methods

JuCCVM - Japanese Center for the
Validation of Alternative Methods



*This workshop will explore how to improve our understanding of key pathways
involved in acute systemic toxicity and applications of this knowledge to
develop new in vitro methods and humane endpoints that will further reduce,
refine, and eventually replace animal use for chemical safety testing.*

February 6-7, 2008 | NIH - Hatcher Conference Center | Bethesda, MD

For more information go to www.niehs.nih.gov/nc3r/nc3r.htm
or www.niehs.nih.gov/nc3r/nc3r.htm

ICCVAM Approver:
• Agency for Toxic Substances
and Hazardous Waste
• Consumer Product Safety Commission
• Department of Agriculture
• Department of Education
• Department of Energy
• National Endowment for the Arts
• National Science Foundation
• Department of the Interior

ICCVAM Approver:
• National Institute of Environmental Health and Safety
• National Institute of General Medical Sciences
• NIH Office of the Director
• National Library of Medicine
• National Library of Health
• National Library of Medicine
• National Library of Medicine
• National Library of Medicine



- *Scientific Workshop on Acute Chemical Safety Testing:
Advancing In Vitro Approaches
and Humane Endpoints for
Systemic Toxicity Evaluations*

- Report published on NICEATM-
ICCVAM website

- NC3Rs addressing workshop
recommendations on using
evident toxicity as a more
humane approach

- [http://iccvam.niehs.nih.gov/
methods/acutetox/toxwksp-
rpt.htm](http://iccvam.niehs.nih.gov/methods/acutetox/toxwksp-rpt.htm)



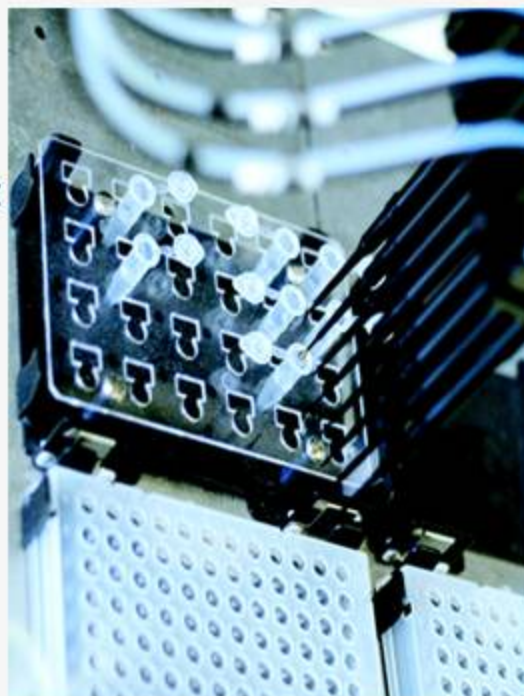
Other Acute Toxicity Test Method Activities

- Neutral Red Uptake Cytotoxicity Tests for Estimating Starting Doses for Acute Oral Toxicity Tests
 - Stokes W, Casati S, Strickland J, Paris M. 2008. Neutral Red Uptake Cytotoxicity Tests for Estimating Starting Doses for Acute Oral Toxicity Tests. *Current Protocols in Toxicology*. May 2008. Suppl. 36: 20.4.1-20.4.20.
- Validation Study: *In vitro* model for human hepatic metabolism and toxicity
 - NICEATM-ICCVAM participation on ECVAM Validation Mgmt. Group
- 3T3 NRU cytotoxicity assays to classify “non-toxic” substances ($LD_{50} > 2000$ mg/kg)
 - ATWG comments on ECVAM validation study
- ATWG
 - ECVAM Liaison - Pilar Prieto
 - JaCVAM - Hajime Kojima



In Vitro Endocrine Disruptor Assays

- International Validation Study: LUMI-CELL®
 - Stably transfected transcriptional activation assay
 - Detects estrogen receptor agonist and antagonist activity
 - NICEATM (lead), ECVAM, and JaCVAM collaboration
- Three labs: U.S., EU, Japan
 - Phase II completed (n=12)
 - Phase III testing ongoing (n=41)
 - Final phase IV, lead lab only (n=25)
- Peer review Spring 2010
- ICCVAM Endocrine Disruptor Working Group
 - ECVAM liaisons: Susanne Bremer, Miriam Jacobs
 - JaCVAM liaison: Hajime Kojima



In Vitro Endocrine Disruptor Assays

- CertiChem, Inc. MCF-7 cell proliferation assay
 - Identifies estrogen receptor agonist and antagonist activity
- Validation Study
 - Phase 1 Testing completed
 - Testing of remaining 41 substances on ICCVAM-recommended minimum list (n=53)



Genetic Toxicity Test Method Activities

- ***In Vitro* Micronucleus Test: OECD draft Test Guideline 487**
 - GTWG provided comments on cytotoxicity procedures and study design
- ***In vivo* rodent Comet assay**
 - JaCVAM international validation study
 - GTWG comments on proposed study plan, protocol, and reference substances
- **Cell transformation assays**
 - GTWG nominated experts for ESAC Peer Review Panel
 - JaCVAM validation study planned, GTWG comments
- GTWG Liaisons:
 - ECVAM: Raphaela Corvi
 - JaCVAM: Hajime Kojima



Dermal Safety Assessment Test Method Activities

- Draft OECD Test Guideline on *In Vitro* Skin Irritation Assays
 - ICCVAM comments provided, 2 rounds
 - Participation in OECD Expert meetings
 - Berlin, October, 2008
 - Washington, June 15-17, 2009
- Updating of OECD Test Guidelines for *In Vitro* Skin Corrosivity Assays
 - TG 430: Transcutaneous Electrical Resistance Test (TER)
 - TG 431: Reconstructed Human Epidermis (RhE) Test Method
 - Updating to include ICCVAM recommended performance standards



Dermal Safety Assessment Test Method Activities

■ Skin irritation assays and corrosivity

- Study underway to determine how *in vitro* dermal irritation test methods (i.e., EpiDerm™ and EPISKIN™) will classify false negative corrosives identified as non-corrosives by *in vitro* corrosivity test methods
 - Current false negative rate for *in vitro* corrosivity assays: 12% to 21%
 - Will confirm extent that new procedure to identify substances that directly reduce MTT will reduce false negative rate in corrosivity assays.

■ ICCVAM Dermal Corrosivity and Irritation Working Group

- ECVAM Liaisons: Valerie Zuang, Tom Cole
- JaCVAM Liaison: Hajime Kojima



Alternative Methods for Biologics Testing: Current & Planned Activities

- Alternatives for vaccine potency testing
 - USDA/MSU study
 - *in vitro* potency tests for Leptospirosis vaccines
 - 2010 International 3Rs Workshop to evaluate the state of the science
- ICCVAM Biologics Working Group
 - ECVAM Liaison: Marlies Halder
 - JaCVAM Liaison: Hajime Kojima



Society of Toxicology Annual Meeting



■ NICEATM-ICCVAM Poster Presentations

- ICCVAM Test Method Recommendations for the Reduced LLNA
- ICCVAM Performance Standards for the Murine Local Lymph Node Assay
- International Validation Study of Estrogen Receptor Transcriptional Activation Test Method
- Scientific Workshop on Acute Chemical Safety Testing; ICCVAM Performance Standards for the LLNA

■ Abstracts and posters viewable on the NICEATM-ICCVAM website

- <http://iccvam.niehs.nih.gov/meetings/SOT09/sotablst.htm>



7th World Congress on Alternatives & Animal Use in the Life Sciences



- Aug 30 - Sep 3, 2009, Rome, Italy
- ICCVAM and NICEATM
 - Chair/co-chair for 5 sessions
 - 8 poster presentations
 - Scientific Organizing Committee, 4 members
 - Program Review Panel, 3 members
- 50th Anniversary Celebration
 - *Principles of Humane Experimental Technique*, William Russell and Rex Burch



Thank you for your attention.

Questions?

Dr. William Stokes
stokes@niehs.nih.gov

<http://iccvam.niehs.nih.gov/>



*The USPHS Commissioned Corps:
Protecting, Promoting, and Advancing the Health and Safety of the Nation*

